EDGEWOOD CHEMICAL BIOLOGICAL CENTER
U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1280

HEADSPACE GAS CHROMATOGRAPHY METHOD FOR STUDIES OF REACTION AND PERMEATION OF VOLATILE AGENTS WITH SOLID MATERIALS

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January 2015

U.S. ARMY RDECOM

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
XX-01-2015	Final	Aug 2011 - Nov 2014

4. TITLE AND SUBTITLE
Headspace Gas Chromatography Method for Studies of Reaction and Permeation of Volatile Agents with Solid Materials

5a. CONTRACT NUMBER
W911SR-11-C-0047

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S
McGarvey, David J. (ECBC); and Creasy, William R. (Leidos)

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRC-M, APG, MD 21010-5424
Leidos, P.O. Box 68, Gunpowder, MD 21010-0068

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1280

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

An analytical chemistry method is described for measuring the reactivity and permeability of fabrics, films, and other solid materials. Headspace gas chromatography (GC) or GC mass spectrometry instrumentation is used. A vial-in-vial method is used, in which the volatile agent is placed in a small inner vial, and the inner vial is capped with a layer of fabric or film to be tested. The agent permeates from the inner vial into an outer headspace vial. The instrument samples the vapor in the outer vial by sampling it and injecting it into the GC for analysis. The presence of agent in the outer vial indicates that it has permeated through the film. Multiple sampling can be used to determine time dependence. Solids can also be used in the headspace vial without the inner vial.

15. SUBJECT TERMS

Headspace gas chromatography	Headspace GC	Self-decontaminating fabric
Reactive fabric	Reactive film	Reactive polymer
HD	Vial-in-vial test	Chemical Weapons agent decontamination
GD		

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Renu B. Rastogi
U	U	U	UU	46	19b. TELEPHONE NUMBER *(include area code)* (410) 436-7545

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PREFACE

The work described in this report was authorized under Contract No. W911SR-11-C-0047. This work was started in August 2011 and completed in November 2014.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

The text of this report is published as received and was not edited by the Technical Releases Office, U.S. Army Edgewood Chemical Biological Center.

This report has been approved for public release.

Acknowledgments

The authors thank Heidi Schreuder-Gibson, Natalie Pomerantz, Nazli El Samaloty, Andra Kirsteins (U.S. Army Natick Soldier Research, Development, and Engineering Center, Warfighter Directorate) and members of the Defense Threat Reduction Agency for support under the Integrated Protective Fabric System project.

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Headspace Gas Chromatography Method for Studies of Reaction and Permeation of Volatile Agents with Solid Materials

ABSTRACT

An analytical chemistry method is described for measuring the reactivity and permeation of fabrics, films, and other solid materials. Headspace GC or GC/MS instrumentation is used. A vial in a vial method is used, in which the volatile agent is placed in a small inner vial, and the inner vial is capped with a layer of fabric or film to be tested. The agent permeates from the inner vial into an outer headspace vial. The instrument samples the vapor in the outer vial by sampling it and injecting it into the GC for analysis. The presence of agent in the outer vial indicates that it has permeated through the film. Multiple sampling can be used to determine time dependence. Reactive fabric or solid samples can be used in the headspace vial without the inner vial.

1.0 SCOPE AND APPLICATION

Headspace gas chromatography (Headspace GC) is used to measure volatile compounds that are in the vapor above a solid or liquid sample in a sealed vial. For this method, the technique is used to measure chemical weapons (CW) agents after they are deposited on fabrics, polymers, or other solid materials. The following attributes of CW agents can be determined: 1) The method can determine whether the reactive analyte is depleted from the vapor. 2) It can detect volatile degradation products. 3) The method can determine the relative vapor pressure of the chemical in the headspace above the solid material to provide qualitative information about the vapor pressure above a sorptive material that does not necessarily promote reaction. 4) The method can measure the permeation of CW agent through a layer of fabric or film by using the “vial in a vial” approach.

Table 1 shows the CW agents that have been tested using the method. Table 2 shows a list of some possible simulant materials that have reactivity that may be similar to CW agents under some conditions.

Table 1: Analytes that have been determined by this method.

CW agent	Chemical name	CAS RN
GB	Diisopropyl methylphosphonofluoridate	107-44-8
GD	Pinacolyl methylphosphonofluoridate	96-64-0
HD	Bis(2-chloroethyl)sulfide	505-60-2
VX*	O-ethyl S-[2-diisopropylaminoethyl] methylphosphonothioate	50782-69-9

*VX has experimental difficulties due to its low volatility that will be discussed in later sections.

Table 2: Simulant Compounds that can be used in this method to mimic the reactivity of CW agents.

Common name	Simulant for agent	Chemical name	CAS RN
DFP	GB or GD	Diisopropyl fluorophosphate	55-91-4
CEES	HD	Chloroethyl ethyl sulfide	693-07-2
Demeton-S*	VX	S-[2-(Ethylthio)ethyl] O,O-diethyl phosphorothioate	126-75-0

*Demeton-S has experimental difficulties due to its low volatility.

CAUTION: The CW agents listed in Table 1 are extremely toxic compounds, and they should be handled only with approved SOPs, protective equipment, hoods, and adequate training to avoid hazards. They are regulated under national laws and international treaties and can only be used at approved facilities. The compounds in Table 2 are significantly less toxic and unregulated, but they are still very hazardous compounds and should be handled with caution.

1.1 Method Limitations

Headspace Gas Chromatography is used to detect volatile compounds in the vapor phase above a solid or liquid sample. The method has been commonly used to detect volatile analytes in water or soil samples for U.S. Environmental Protection Agency methods.[1] As such, it is limited to volatile compounds. Many degradation products of CW agents are not volatile, so the degradation products may not be detected. Reactivity or vapor pressure reduction will be measured by the decrease in the analyte signal, rather than by comparison of the analyte to product signal to obtain mass balance of reactants and products. It may be possible to use a different analytical method to obtain information about the nonvolatile compounds. For example, the fabric or solid sample that is used in this test can be solvent extracted, and the solvent can be analyzed by a liquid injection method to look for degradation products.

The measurement is made between the difference in signal between a spiked blank sample and a reactive sample. As a result, sensitivity depends on the dynamic range between the highest amount of CW agent that can be spiked without saturating the detector, and the lowest amount that can be detected reliably. Sensitivity also depends on the volatility of the agent that is being tested, or the inherent vapor pressure of the agent at a particular temperature. For GD or HD, the maximum spike amount may be <100 µg of agent spiked on a 1 cm^2 fabric sample in a 10-20 ml headspace vial. The minimum detection is <1 µg. For VX, the vapor pressure is much lower, which limits the vapor exposure for the agent to fabrics and the sensitivity of the detection. However, this information is included for guidance only, since detection limits are highly matrix dependent and are not always achievable. Detection limits should be determined for each matrix. The method can be optimized for lower absolute detection limits by adjusting the detector conditions or by using SPME sampling of the headspace vial.

The method is limited to analytes that have enough volatility to be sampled in the headspace. The instrument is designed so that the headspace vial is heated during sampling. The heating can be increased to >100°C, which increases the vapor pressure of the analytes. However, the

reactive material is also heated at the same time. Heating the material may cause the reaction rate to change, causing the material to appear to be more reactive than it actually is at room temperature. For this reason, testing of low volatility compounds must be considered unreliable, unless method validation is performed.

Prior to employing this method, analysts are advised to consult laboratory requirements for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statements for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

Use of this method is restricted to use by, or under supervision of, personnel appropriately experienced and trained in the use of gas chromatography. Each analyst must demonstrate the ability to generate acceptable results with this method. Method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

This report is a guidance method that contains general information on how to perform an analytical procedure or technique. It is distinguished from a detailed Standard Operating Procedure (SOP) for a specific project application, or analysis reports that report specific data and interpretation. The performance data included in a method are for guidance purposes only, and are not necessarily acceptable for absolute QC acceptance criteria.

2.0 SUMMARY OF METHOD

2.1. Preparation of material

2.1.1. Fabric reactivity: A known quantity of fabric material is cut from a roll or swatch and placed in a headspace vial. Commercial headspace vials are typically 10 or 20 ml in volume. An amount of 1 cm × 1 cm can be used for simple comparison. However, the amount of fabric is only limited by the amount that will fit in the selected headspace vial. The amount of fabric can be determined by area or by weight. These are recorded so the same amount of a suitable blank fabric is used for comparison.

2.1.2. Polymer or other solid material: A suitable amount of solid, powdered, or chunky solid is placed in the headspace vial. The solid is weighed. The solid should be consolidated so that it can be spiked. If necessary, a smaller glass container can be placed inside the headspace vial, such as a GC vial insert, to contain the solid material so that it can be spiked more directly.

2.1.3. Film permeation: A circle of fabric or film is cut using a hole punch. The circle is placed in a GC vial cap in place of the silicone polymer seal that is typically used on a GC vial

cap. A diagram is given in a later section. The cap is crimped to seal the circle of film to the edge of the small vial. The small GC vial is placed inside the larger headspace vial.

2.1.4. Unreactive reference preparation: The most reliable type of determination is a direct comparison between a material that is reactive and a comparable type of material that is unreactive. For example, if a fabric is chemically treated to be reactive, a comparison of the chemically treated material to the same untreated material is used. For some polymers, the polymer composition is inherently reactive, so this approach may not be possible. But the most defensible result will be a blank sample of material that is prepared in the same way as the reactive sample, either a fabric, polymer, or other material. It is also important to pay attention to the density of the weave and porosity of a fabric. A loose weave can allow more direct diffusion through the fabric. Unreactive reference materials should have the same physical weave and porosity compared to the reactive material.

2.2. Spiking the sample: A neat or dilute sample of CW agent or simulant is obtained. A neat standard should only be used for a very reactive or very absorbent material, since otherwise it will likely saturate the detector. Dilute standards can be used in any appropriate volatile solvent that doesn't affect the material to be studied. It is preferable to allow the solvent to evaporate before measurements begin. The fabric, polymer, or solid material is spiked using a known weight or volume of the solution or standard. Solvent is allowed sufficient time to evaporate, then the headspace vial is capped. Unreactive control fabrics are spiked exactly the same as the samples. For the film permeation experiment, the spike is placed inside a small inner vial, and the small vial is capped with the modified vial cap (Section 2.1.3), and then the small vial is placed in the larger headspace vial that is also capped.

2.3. Reaction time: Allow the sealed vials to sit at room temperature for the necessary reaction time.

2.4. Sample analysis: Analyze the vial headspace using a headspace GC instrument. A specific model of instrument and instrument parameters are included, but a number of instrument models and configurations can be used.

2.4.1 Method sensitivity: The data results are usually reported in terms of a ratio between the unreactive control and the reactive sample. In order to have a meaningful ratio, signals must be nonzero and not saturated for both the control and the sample. The ratio is limited by the dynamic range of the instrument. It may be necessary to adjust the analytical method to make it more or less sensitive, since the amount of signal may not be predictable in advance simply from the amount of agent that is spiked. If the control material produces too much signal, the signal may saturate, and the result cannot be used for a meaningful ratio. On the other hand, if the reactive sample gives a signal that is too low, the result may be very uncertain due to errors or it may not be distinguishable from zero. As a result, it may be necessary to adjust the sensitivity of the method using trial runs to make sure that both samples are in range of the detector. For GC methods, there are many parameters that can be used to

adjust the sensitivity of the response: split vs. splitless injections, injection volume, and detector gain.

2.4.2 Linear vs. nonlinear detector response: If the detector gives a linear response, a ratio between control and reactive samples can be obtained from the ratio of the signal. However, if the detector response is nonlinear, such a ratio may not be valid. It is necessary or advisable to perform an instrument calibration before sample analysis. Using the calibration, signal can be transformed to a concentration using the calibration equation, and then the ratio of the control and reactive samples can be determined from the concentration. As discussed in section 2.4.1, the range of the calibration standards that are needed may have to be determined using trial runs of actual controls and samples.

3.0 DEFINITIONS

Headspace Gas Chromatography: The analytical chemistry instrumental technique for sampling and analyzing the chemicals in the vapor layer of a sealed vial above a solid or liquid sample. The vapor is analyzed by chromatography to separate the target analyte from interferences that may be present in a liquid extraction or direct analysis of the solid or liquid.

Mass Spectrometry (MS): Instrument that is used as a detector for GC. The instrument introduces a vapor stream into a vacuum chamber, and the analyte in the vapor is ionized to produce characteristic ions using a number of different ionization methods. The ions are mass analyzed and detected to produce a signal that is proportional to the amount of analyte.

Flame Photometric Detector (FPD): GC detector that detects analytes by burning the vapor stream in a hydrogen/air flame to produce fluorescent emission from characteristic species in the flame. The light emission is amplified by a photomultiplier. The FPD can be designed to operate in a pulsed mode as a pulsed FPD.

Traditional Chemical Weapons Agent or **Chemical Warfare Agent (CWA)**: The toxic chemicals that were stockpiled either by the U.S., Soviet Union, or other countries. These compounds are often referred to by a one or two letter code, such as GB, GD, HD, or L. These chemicals have been banned by the Chemical Weapons Convention (CWC) Treaty with certain specified exceptions.

Simulant: A compound with less toxicity than a CWA that is used to simulate the properties of the CWA. Some simulants are used to model dispersal in the environment, so they require toxicity that is so low that they can be released into the environment. For reactivity studies, compounds must be similar enough to the CWA compounds that they typically have some toxicity, but they can be used with lower hazard than CWAs.

Refer to scientific literature and the manufacturer's instructions for other definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Typical headspace GC/MS method analyses are subject to interferences for a number of volatile compounds if they have GC characteristics that are similar to the analyte. This method is less subject to the problems of interferences than most headspace methods because the analyte compounds are unlikely to be contaminates of the vapor of a laboratory, and therefore less likely to find their way into samples, than typical volatile organic compounds. However, caution is still needed to avoid the contamination of samples with the analytes. Samples can be contaminated by diffusion of volatile organics through the septum seal of the sample vial during shipment and storage, although this problem is unlikely because these samples will be analyzed near the laboratory that they are prepared. If the samples are transported, a trip blank prepared from an appropriate organic-free matrix and sample container, and carried through sampling and handling protocols, serves as a check on such contamination. The trip blank is only necessary if the samples are being transferred after preparation.

4.2 The sample matrix itself can cause interferences by one of several processes or a combination of these processes. These include, but are not necessarily limited to, the absorption potential of the solid and the actual composition of the solid. Some solids may outgas volatile compounds that can interfere with the detection of the target analyte. Some solids inhibit the partitioning of the volatile target analytes into the headspace, therefore, recoveries will be low. This effect may be a desired attribute of the solid material. The analyst should be aware that the low vapor pressure of the target analyte may not indicate that the analyte has been reactively destroyed, only that it has a low partition into the vapor. It is possible to use a nonreactive surrogate compound to address some aspects of this issue.

4.3 Contamination by carryover can occur whenever high-concentration and low-concentration samples are analyzed sequentially. Where practical, samples with unusually high concentrations of analytes should be followed by an analysis of unspiked blanks to check for cross-contamination. If the target compounds present in an unusually concentrated sample are also found to be present in the subsequent samples, the analyst must demonstrate that the compounds are not due to carryover. Conversely, if those target compounds are not present in the subsequent sample, then the analysis of blanks is not necessary.

4.4 The laboratory where volatiles analysis is performed should be completely free of target analytes. If unspiked blank samples contain compounds that saturate the detector or interfere with the observation window for the target analyte, then other organic solvents in the laboratory may have to be eliminated, since volatile organics can lead to random background levels, so precautions must be taken.

5.0 SAFETY

The CW agents listed in Table 1 are extremely toxic compounds, and they should be handled only with approved SOPs, protective equipment, fume hoods, and adequate training to avoid

hazards. They are regulated under national laws and international treaties and can only be used at approved facilities. The quantities of analyte that are used in these experiments are less than the typical lethal dose, but they are a significant fraction of the toxic dose for an adult human.

The compounds in Table 2 are significantly less toxic and unregulated, but they are still very hazardous compounds and should be handled with caution and with approved safety procedures.

Workers who are uninformed about and unprotected from toxic symptoms should not be in the lab when toxic compounds are in use, since there can be a significant vapor hazard in case of spillage outside of a fume hood or in case of power failure. MSDSs should be consulted for toxicity information and personal protective equipment.

During analysis, the sample vials should be tightly capped before removing them from the fume hood. The analytical instrument is typically not installed in a hood. Therefore, for samples with high spiking levels and depending on the toxicity of the compound, it may be advisable to wear respiratory protection during the sample analysis if the lab doesn't have adequate air flow.

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this method is for illustrative purposes only, and does not constitute an endorsement or exclusive recommendation for use. The products and instrument settings cited represent those products and settings used during method development or subsequently evaluated. Glassware, reagents, supplies, equipment, and settings other than those listed in this method may be employed provided that method performance appropriate for the intended application has been demonstrated with appropriate blanks and spiked QC samples.

6.1 Headspace Containers are glass, 10-ml or 20-ml vials that are equipped with a polytetrafluoroethylene (PTFE)-lined septum and are compatible with the analytical system. (For example, VWR screw cap vials, 20 ml Screw-Top Headspace, part number 89047-694, with screw caps part number 97035-458, or equivalent vials.) 20-ml vials are recommended to provide more space for the inner vial. Vials of 10 ml volume or other sizes may be employed, provided that they can be hermetically sealed and equipped with a suitable septum.

6.2 Headspace System - This method was developed using a totally automated equilibrium headspace analyzer, Gerstel MPS2 autosampler with Static Headspace option, which uses a heated agitator and heated gas syringe to equilibrate and sample the vial, followed by injection into the Gerstel injection port on a Agilent Gas Chromatograph/Mass Spectrometer (Gerstel, Inc., 701 Digital Drive Suite J, Linthicum, MD 21090). The Gerstel injection port vents the excess gas so it does not flow through the GC column. Another system is a CTC CombiPAL headspace autosampler, which is similar to the Gerstel MPS2. Another type of GC is a Varian CP-3800 GC with various detectors, including a mass spectrometer or a pulsed FPD. Similar systems are available from several other commercial sources. If other headspace systems and determinative methods are utilized, it is recommended that the manufacturer's headspace operating conditions be followed, provided that they are appropriate for the determinative method to be employed. The system used must meet the following specifications:

6.2.1 The system must be capable of holding samples at elevated temperatures and establishing a reproducible equilibrium between a wide variety of sample types and the headspace.

6.2.2 The system must be capable of accurately transferring a representative portion of the headspace into a gas chromatograph fitted with a capillary column. This must be accomplished without adversely affecting the chromatography or the detector. This can be done using manual injections, but an automated system should provide more reproducibility. Solid Phase Microextraction can also be used, with appropriate extraction fibers and desorption conditions, but extra care must be taken to keep the conditions as reproducible as possible and avoid artifacts.

6.2.3 The operating conditions listed in Sec. 11.0 are those selected for the equipment used in developing this method. Other equipment and conditions may be employed, provided that the laboratory demonstrates performance for the analytes of interest using the determinative method appropriate for the intended application.

6.3. For humidifying samples, a variable humidity chamber can be used. For example, a Thunder Scientific humidity chamber (or equivalent) is used to humidify the fabrics or materials before spiking. See Section 8.

6.4. For film or fabric permeation determinations, the following 0.8 ml GC vials are used, or equivalent: Perkin Elmer 0.8 ml glass vials, Part No. N930-1069, (also available from Chromacol, P. O. Box 293, Trumbull, CT 06611), and associated aluminum crimp caps (Chromacol Part Number 5110-08, 8 mm SEAL with 10 mil PTFE, or Thomas Scientific Cat. No. 2701S01, Aluminum Seal with Septa, or equivalent). Aluminum caps require a correct size of crimper tool, such as Chromacol Part Number 9300-08, 8 mm Hand Operated Crimper, or equivalent. (It is necessary to use 0.8 ml glass vials with standard 10 ml or 20 ml headspace vials, since the small vials must fit inside the headspace vials. For the standard 10 ml or 20 ml headspace vials, a common 2-ml GC autosampler vials will not fit inside.) Plastic snap caps

can be placed on vials by hand without a crimper tool, but some experiments have indicated that the plastic caps may absorb analyte and affect the permeation measurement.

7.0 REAGENTS AND STANDARDS

7.1. Fabrics and materials can be engineered for reactions with particular agents. Specific tests may be selected depending on the type of agent that the fabric is designed for. Testing of the agents or simulants depends on the capability and certification of the laboratory for agent testing. The following agents or simulants can be selected as appropriate:

7.2. The following CW agents are distributed by the Chemical Agent Standard Analytical Reference Material (CASARM) Program, Edgewood Chemical Biological Center, Aberdeen Proving Ground-Edgewood Area, MD:

GB, Isopropyl methylphosphonofluoridate, CAS RN 107-44-8 (>95% purity), $C_4H_{10}FO_2P$.
GD, Pinacolyl methylphosphonofluoridate, CAS RN 96-64-0 (>95% purity), $C_7H_{18}FO_2P$.
HD, Bis(2-chloroethyl)sulfide, CAS RN 505-60-2 (>95% purity), $C_4H_8Cl_2S$.
VX, O-ethyl S-[2-diisopropylaminoethyl] methylphosphonothioate, CAS RN 50782-69-9 (>90% purity), $C_{11}H_{26}NO_2PS$.

CAUTION: The CW agents are extremely toxic compounds, and they should be handled only with approved SOPs, protective equipment, hoods, and adequate training to avoid hazards. They are regulated under national laws and international treaties and can only be used at approved facilities.

7.3. The following simulants are purchased commercially:

DFP, (simulant for GB or GD), Diisopropyl fluorophosphate, CAS RN 55-91-4 (>95% purity), $C_6H_{14}PO_3F$.
CEES, (simulant for HD), Chloroethyl ethyl sulfide, CAS RN 693-07-2 (>95% purity), C_4H_9ClS.
Demeton-S, (simulant for VX), S-[2-(Ethylthio)ethyl] O,O-diethyl phosphorothioate, CAS RN 126-75-0 (>95% purity), $C_8H_{19}O_3PS_2$.

CAUTION: These compounds are significantly less toxic than the CW agents and they are unregulated, but they are still very hazardous compounds and should be handled with caution.

7.4. All reagents can be spiked on the fabric or materials as neat compounds or as dilute solutions. The solvents for the dilutions should be tested to make sure that they are compatible with the fabric or solid sample. The CW agents are stored under refrigeration in double containment. Agents must be secured in locked storage under inventory control.

Reagent-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

7.4.1 Calibration spiking solutions – Prepare five, or more, spiking solutions in methanol, or other compatible solvent, that contain all the target analytes and the surrogate standards. The concentrations of the calibration solutions should be such that the addition to the 10 ml or 20 ml vials will bracket the analytical range that is required for the control and reactive samples.

7.4.2 Internal and surrogate standards – For quantitative determinations, follow the recommendations of the determinative methods for the selection of internal and surrogate standards. External standard calibration may be preferred and the internal standard is omitted. The concentration may vary depending on the relative sensitivity of the GC system or any other determinative method that is utilized.

7.5 Blank Preparation – Place blank material in an empty vial. Inject the necessary amounts of the internal standards and surrogate compounds in the headspace vial, and seal the vial. Place it in the autosampler and analyze in the same manner as an unknown sample. Analyzing the blank in this way will indicate possible problems with the autosampler as well as the headspace device.

7.6 Preparation of Calibration Standards - Prepare calibration standards in the same manner as the blanks (Sec. 7.5), adding the standard spiking solutions prepared in Sec. 7.4.1 in the same manner that the internal standards and surrogates are added.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1. Sample preparation: Some fabrics or materials must be treated to specifications before reaction. For example, humidity conditions within a protective suit can be high when the wearer is sealed inside, so tests for reactivity of the suit material are done at high humidity for comparison. Nerve agents typically undergo hydrolysis, so the reactivity can be higher at high humidity.

The fabric or material producer and project manager must be consulted for the appropriate testing conditions to meet the requirements of the customer.

To condition the fabric or material to a specified humidity, the sample can be humidified for at least 3 days in a variable humidity chamber, for example Thunder Scientific.

Alternately, the fabric or material can be placed in a humidifying bottle. A bottle containing distilled, deionized water has a humidity of 100% relative humidity (RH). A bottle containing a saturated salt solutions can produce lower relative humidity. Test material should be exposed only to humidified air in the bottle, not to the liquid water or solution.

For best results, a fan should be in the bottle to circulate the air. If there is no fan, longer times may be needed to totally equilibrate the material. A humidity meter should be used to monitor the humidity inside the bottle. For example, a Fisher Scientific traceable remote alarm RH/temperature monitor, part number 14-649-84 (or equivalent), can be used to measure humidity inside a bottle.

8.2. Comparable unreactive material: For best results, a reactive material is directly compared to a corresponding unreactive material to test the effectiveness of the reactive treatment. If a fabric is chemically treated to be reactive, the unreactive material is the same fabric that is untreated. For some polymers, the polymer composition is inherently reactive, so an unreactive material is a polymer with similar composition and porosity but which is inert. For film permeation determinations, it is recommended that both an impermeable and an unreactive reference sample are tested. The original GC vial septum (PTFE or silicone polymer) can be used as the impermeable reference, and the unreactive reference can be the untreated film. The unreactive material and impermeable reference, if used, are prepared in a way identical to the reactive material.

9.0 QUALITY CONTROL

Any effort involving the collection of analytical data that goes beyond research and development should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection. Refer to references for guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over technique-specific criteria.

9.1 Routine stability testing

The QC sensitivity measurement can be done by preparing a vial with a standard quantity of a reagent or standard surrogate compound that can be detected with similar sensitivity as the reagent of interest. However, preparing a standard for long-term stability checks has been a problem for this method. The problem is illustrated in Figure 1. Volatile compounds like triethylphosphate or trimethylphosphate have an exponential decrease in signal when they are

in the headspace vial. The reason for this decrease has not been conclusively identified, but it occurs for volatile compounds that completely evaporate in the vial. The compounds may be escaping from the vial, or being absorbed in the septum or on the glass vial surface. Because of this large decrease in signal, the standard made from a volatile compound in an empty headspace vial is not effective to check for long-term stability of the instrument.

The triisopropylphosphate shows much more stable signal. This is caused by the lower volatility of the compound. A drop of liquid remains in the vial, so as the vapor decreases, more of the liquid evaporates, which maintains a constant headspace concentration. The signal decreases in the vial after 600 min. when all the liquid has evaporated. As a result of this behavior, a selected low volatility compound can be used as a stability check. However, if the compound has volatility that is too low, the signal will be too low to be useful.

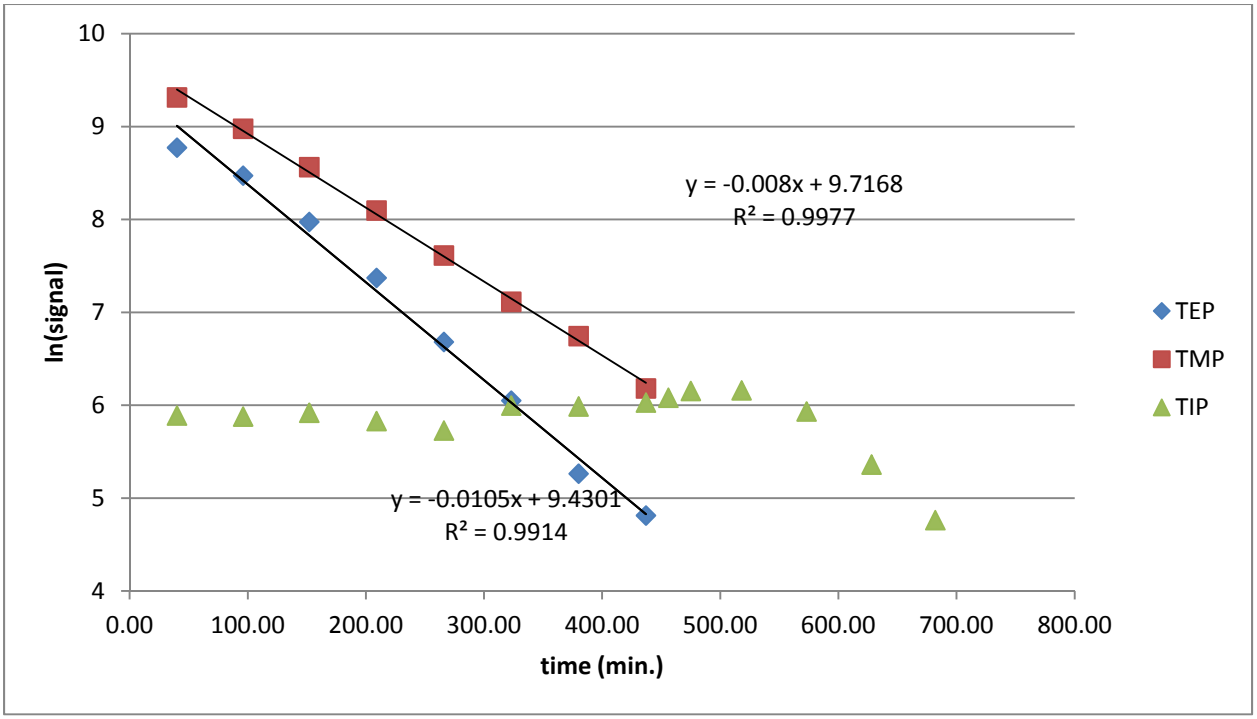

Figure 1: Decrease in signal for triethylphosphate (TEP), trimethylphosphate (TMP), and triisopropylphosphate (TIP) as a function of time, plotted on a log scale. Each was added to a separate headspace vial as 10 µl of neat liquid.

Selection of a compound with a particular volatility doesn't help to find a stable check standard for an arbitrary volatile compound. Another alternative was found. HD, bis(2-chloroethyl) sulfide, is volatile enough to give the same behavior as TEP. A standard solution of HD was made in decane at a concentration of 1.3 mg/ml. This solution has a partial pressure of HD that is 1.4 ng/ml at 25°C in the headspace vial.[2] This concentration provides a convenient amount of HD for the instrument. Since the liquid decane solution can be put in the headspace vial, the HD is replenished in the vapor by the solution, providing a stable vapor concentration.

Figure 2 shows the signal from the GC/pulsed FPD detector for the same solution of 1.3 mg/ml HD in decane collected over 17 days. The relative standard deviation is 33%. Since the FPD detector has a quadratic response to sulfur, the calibrated amount of HD has an RSD of 26%. Although the standard deviation is significant, it is much more appropriate for a stability check than the exponentially decreasing signal.

The decane solution worked for HD, but it was found that solutions of acetonitrile, chloroform, and dimethylsulfoxide did not perform well. It is possible that these solvents do not follow the ideal partial pressure law with HD.

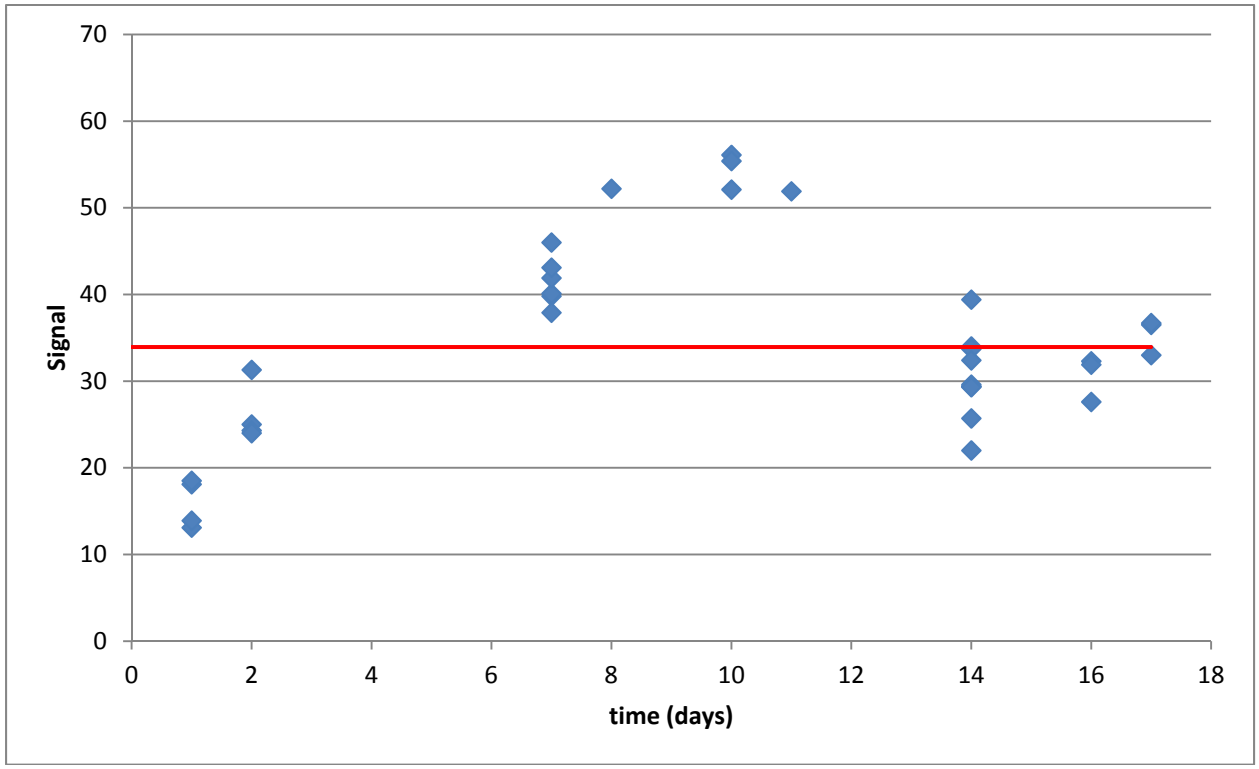

Figure 2: Plot of pulsed FPD signal for the same 1.3 mg/ml solution of HD in decane, over the course of 17 days.

If signal response is found to be less stable than this benchmark during routine sample analysis, for QC purposes, the instrument manuals or service representatives can be consulted to restore the system performance.

9.2 Purity of standard reagents

QC testing is done periodically on the neat CW agent standards or dilute standards to check the purity. Validating the purity of the stock standard can be done by NMR to determine the purity as a weight percent by using an internal standard.[3] It can be done by referencing the response of the CW agent to a stable compound at a known concentration. Since CW compounds can be

reactive with water vapor, the purity must be checked periodically to determine that any reaction is due to reaction with the test material and not reaction prior to spiking. Alternately, a new standard can be obtained periodically from CASARM.

9.3 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with a method analysis by generating data of acceptable accuracy and precision for target analytes in a clean matrix. Proficiency testing includes a demonstration of knowledge of the operator and proper operation of the instrument. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made.

9.4 Check for interferences

Before processing any samples, the analyst should demonstrate that the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. Each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If a peak is observed that would prevent the determination of an analyte, determine the source and eliminate it, if possible, before processing the samples. The blanks should be carried through all stages of sample preparation and analysis.

9.5 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, method sensitivity). At a minimum, this should include the analysis of QC samples including a method blank, a matrix spike, and a duplicate in each analytical batch. Any method blanks, matrix spike samples, and replicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

If a sample of the unreactive fabric or material is available for use, the unreactive equivalent material can be used as a blank or control material. In this case, the comparison between the control and the reactive material is used to show the extra reactivity of the reactive material. The control samples show the baseline reactivity which may be due to ambient humidity and air.

The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on knowledge of the samples in the sample batch and the project requirements. If samples are expected to contain target analytes, laboratories may use a matrix spike and a duplicate analysis of an unspiked sample. If samples are not expected to contain target analytes, the laboratories should use a matrix spike and matrix spike duplicate pair.

For measurement of kinetics, a QC measurement is done at the beginning and ending of the analysis to determine that the signal response is stable. This is particularly important if the

kinetics is determined by the decrease in absolute signal of a peak when product peaks cannot be detected.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Detector calibration

Calibration of the detector can be done by liquid injections of dilute solutions of the analyte. This can easily be done with an autosampler, if the syringe and injection conditions can be changed to perform a liquid injection. If manual injections are done, it is possible to do manual injections of liquid standards. Figure 3 shows a calibration curve of a pulsed FPD detector using liquid injections of solutions of HD from 0.5 to 5 µg/ml in concentration using a 1 µl injection. Since the PFD detector has a quadratic signal response to S, the square root of the signal is plotted.

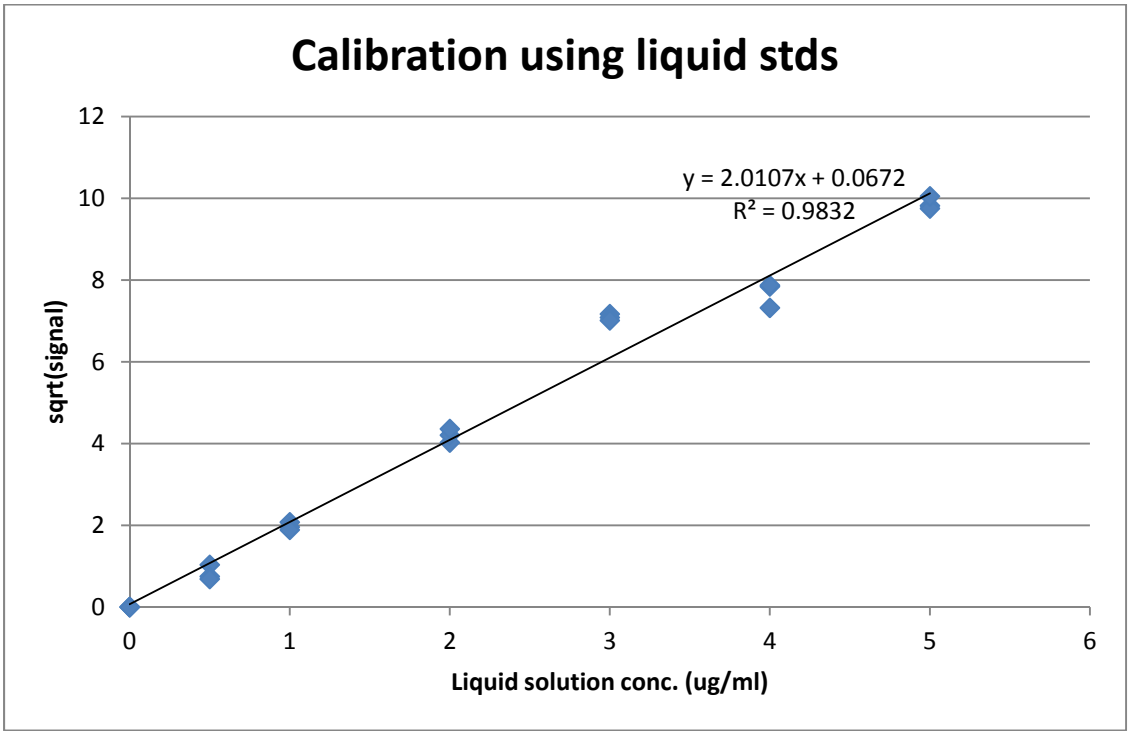

Figure 3: Calibration curve of HD using liquid injections. The solutions are in concentrations of µg/ml with a 1-µl injection volume. The detection was done with a pulsed FPD detector, which has a quadratic response to sulfur, so the square root of the signal is plotted.

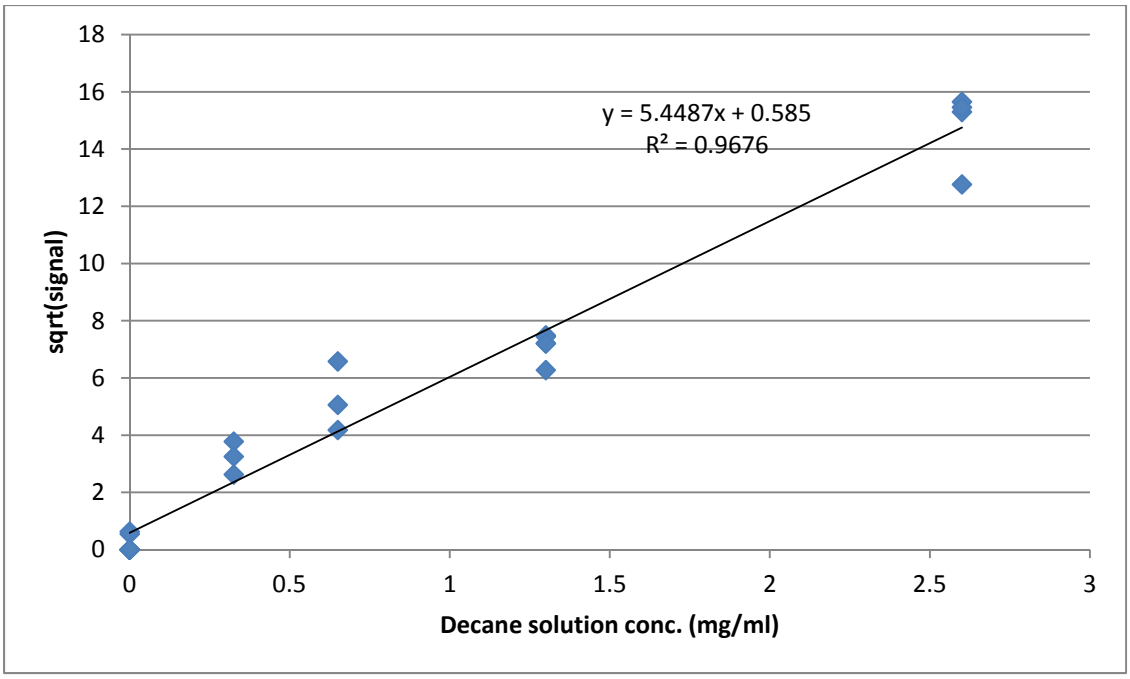

Figure 4: Calibration curve of HD using headspace vapor injections. The solutions are in concentrations of mg/ml, and 0.5 ml of vapor is injected on the GC at 40°C. The detection was done with a pulsed FPD detector, which has a quadratic response to sulfur, so the square root of the signal is plotted.

The signal response can also be calibrated with solutions of HD in decane of the same type that was used in Figure 2. The calibration curve is illustrated in Figure 4.

The headspace solution calibration can be done with the same syringe and injection conditions as are used for the headspace samples. For this example, injection volumes of 0.5 ml were used.

10.2 Conversion between the two types of calibration

For a liquid injection, the amount of analyte that is injected on column is given by:

Amt. analyte = (injection volume) × (standard concentration)

For headspace injection, the headspace vapor concentration must be determined. For standards of HD, the vapor pressure can be determined from the literature,[2] and it is 0.106 torr at 25°C and 0.336 torr at 40°C. Partial pressures for ideal solutions are determined from the mole fraction of solute in solvent times the vapor pressure for the neat liquid.

For 1.3 mg/ml HD in decane,

Moles of HD = wt. HD/MW HD = 1.3 mg/159.08 = 8.2×10^{-6} moles

Moles decane = vol. × density/MW = 1 ml × 0.73 g/ml/142.3 g/mole

= 0.0051 moles

Mole fraction HD = 0.0016

Partial pressure = 0.336 torr × 0.0016 = 0.00054 torr (at 40°C)

Using the ideal gas law with R in the correct units (62.36 L-torr-K^{-1}-$mole^{-1}$),

$n = PV/RT = 2.75 \times 10^{-11}$ moles/ml = 4.4 ng HD/ml vapor

An injection of 0.5 ml of vapor gives 2.2 ng of HD on column, for a 1.3 mg/ml solution of HD in decane that has equilibrated vapor at 40 °C in a headspace vial, independent of the volume of the headspace vial for saturated, equilibrated vapor. By comparing the calibration curves in Figure 3 and Figure 4, the measured amount of HD for the 1.3 mg/ml solution corresponds to the signal for 3.78 ng. This is reasonable agreement for the different measurements. The difference may reflect different efficiencies for injecting and trapping HD on the GC column.

10.3 Standardization

In order to check the standardization of permeation, it would be necessary to have a test fabric or film with a known amount of permeation to use to check the results of the method. This would allow the permeation to be tested with a known standard. Unfortunately, for the course of the IPFS project, a standard with a known permeation was not available. Results are given in relative terms between a test material and a reference material.

For further information, see Sec. 11 for information on calibration and standardization of particular materials and agents.

11.0 PROCEDURE

11.1 Pretreatment of material: Some fabrics or materials must be treated to specifications before reaction. For example, tests for reactivity may be done at high humidity. Nerve agents typically undergo hydrolysis, so the reactivity can be higher when the materials are conditioned at high humidity so a significant amount of moisture is absorbed. The fabric or material producer and project manager can be consulted for the appropriate testing conditions to meet the requirements of the final application. To condition the fabric or material to a specified humidity such as 80% or 95% RH, the sample is humidified for at least 3 days in a humidity chamber. After humidification, the samples can be handled at ambient humidity to prepare the vials.

11.2. Preparation of material. Sample preparation is different for three different types of experiments: fabric reactivity, polymer/solid material reactivity, or film permeation. Each experiment requires the preparation of a similar type of unreactive sample, as well as QC standards.

11.2.1. Fabric reactivity vial preparation: A known quantity of reactive fabric material is cut from a roll or swatch that was uniformly treated. It is placed in a headspace vial. Commercial headspace vials are typically 10 or 20 ml in volume. An amount of 1 cm × 1 cm can be used for simple comparison of spike amount to area. However, the amount of fabric is

only limited by the amount that will fit in the selected headspace vial. The amount of fabric is determined by area or by weight. Record the fabric area or weight. The same amount of a suitable unreactive fabric is used for comparison.

11.2.2. Polymer or other solid material vial preparation: A suitable amount of powdered or chunky solid material is placed in the headspace vial. If necessary, a smaller glass container, such as a GC vial insert, can be placed inside the headspace vial, to contain the solid material so that it can be spiked more directly without losing the spiked material on the glass vial. The solid is weighed by difference.

11.2.3. Film permeation vial preparation (also referred to as the vial-in-vial samples): A circle of fabric or film to be tested is cut in a circle using a hole punch. A hole punch is recommended such as 1280ST- 6 PC Hollow Steel Punch Set, www.generaltools.com. The minimum circle size is 8 mm in diameter to cover the inner vial, but a larger circle of 5/16" is preferable to prevent it from slipping to the side of the cap when it is crimped. The circle is placed in a GC vial cap, preferably an aluminum crimp cap, for a 0.8 ml GC vial. The material replaces the silicone or PTFE polymer seal that is typically used to seal a GC vial. Take care to make sure that the circle is not so big that it will not be uniformly in contact with the lip of the small vial. Also, make sure there are no fringes or fraying that will interfere with uniform contact. Leave the caps off the GC vial and off the headspace vial until spiking. Some users prefer to also use a ring of Teflon as a seal under the crimp cap.

11.3. Unreactive material vial preparation: Obtain a comparable unreactive material. For example, if a fabric is chemically treated to be reactive, the unreactive material is the same fabric that is untreated material. For some polymers, the polymer composition is inherently reactive, so an unreactive material is a polymer with similar composition and porosity but which is inert. For film permeation determinations, it is recommended that both an impermeable and an unreactive reference sample are tested. The original GC vial septum (PTFE or silicone polymer) can be used as the impermeable reference, and the unreactive reference can be the untreated film. The unreactive material and impermeable reference, if used, are prepared in a way identical to the reactive material. If no unreactive material can be obtained, the lack of comparison is documented in the report on the results.

11.4. Calculate the spike amount: A neat or dilute sample of CW agent or simulant is obtained. The required spike weight of CW agent or simulant is calculated based on the required dose (in weight per area, in mg/cm^2) using the area of the fabric, or based on a weight/weight dose, using the weight of the material. The maximum weight per area dose that is required is typically 1 mg/cm^2 (or 10 g/m^2) or less. A neat standard should only be used for a very reactive or very absorbent material, since otherwise it will likely saturate the detector. Dilute standards can be made in any appropriate volatile solvent that is compatible with the material. The volume of the spike solution of a dilute standard is calculated from the concentration.

11.5. Spike the material: The fabric, polymer, or solid material is spiked using the calculated volume of the spiking solution or standard. Solvent is allowed sufficient time to evaporate, then the headspace vial is capped. For the film permeation experiment, the spike is placed inside a small inner vial, and the small vial is capped with the modified vial cap, and then the small vial is placed in the larger headspace vial that is also capped. The configuration of the final assembled sample is shown in Figure 5. The photo of the parts of the film permeation samples is shown in Figure 6.

11.6. Unreactive samples (and impermeable reference films, if used) are spiked to the same weight per area or weigh per weight dose as the samples.

11.7. Blank samples are prepared from the same amount of reactive materials, but are not spiked with the CW agent or simulant.

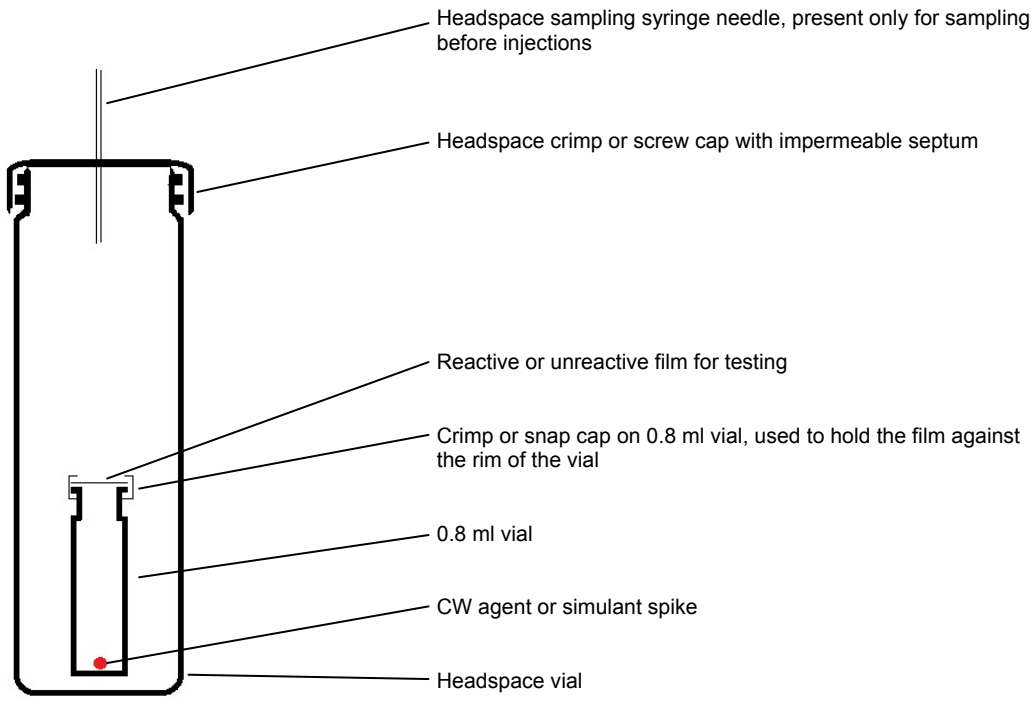

Figure 5: Final configuration of the vials for measurement of film permeation.

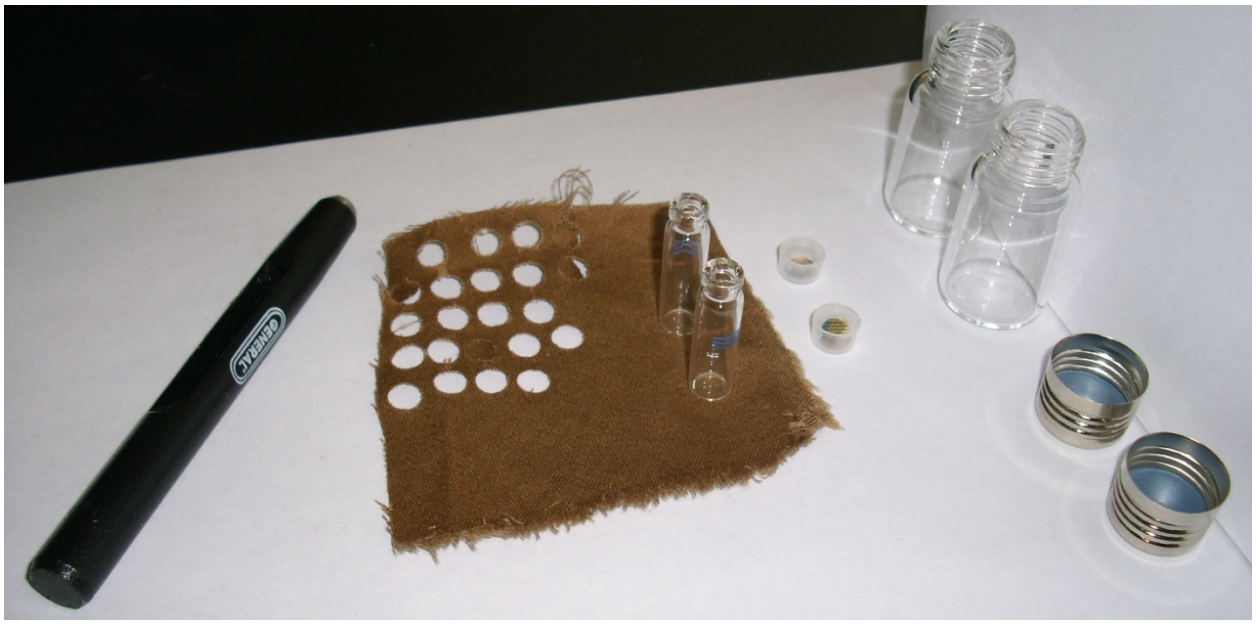

Figure 6: Photo of the hole punch, fabric, small inner vials, modified caps, headspace vials, and headspace vial caps used for the preparation of vial-in-vial permeation test samples.

11.8. Reaction time: Allow the sealed vials to sit at room temperature for the necessary reaction time. For multiple kinetic time points, several identical vials can be prepared at the same time and one is analyzed at each time point. Samples can be rerun multiple times using an autosampler sequence.

11.9. Calibration or QC standards:

11.9.1. For quantitative determinations, an external calibration curve is generated. The vials are analyzed with the same instrument conditions as the sample vials, unless they are done using liquid injections (see Section 10). A calibration curve is generated from the signal for the analyte vs. the amount of analyte, at a particular vial equilibration temperature and instrument conditions.

11.9.2. For qualitative determinations or relative comparisons of reactive and unreactive samples, one QC vial is used to test the performance of the instrument. The amount of analyte in the vial is similar enough to the amount in the spiked samples that the detector isn't saturated. The analysis for the unreactive material samples may be sufficient as a QC analysis.

11.9.3. Avoiding saturation of the instrument response: For high spike levels on fabrics or materials, the instrument signal response may saturate. In this case, there may not appear to be a difference between reactive and unreactive samples even if there is a real difference, if both are above the saturation amount. In cases for which a high signal response is observed, several calibration standards should be run at and below the highest amount that is expected, to determine the amount that corresponds to signal saturation. If the instrument is saturating, the

method can be changed to decrease the sensitivity, for example by decreasing the injection volume or increasing the split ratio of the GC injection. When acceptable parameters are obtained, the calibration standards must all be rerun.

11.10. Sample analysis: Samples, blanks, and QC standards, and calibration standards in headspace vials are analyzed using a headspace GC instrument using the same conditions. This method was developed using a totally automated equilibrium headspace analyzer, Gerstel MPS2 autosampler with Static Headspace option, which uses a heated agitator and heated gas syringe to equilibrate and sample the vial, followed by injection into the injection port on a Agilent Gas Chromatograph/Mass Spectrometer (Gerstel, Inc., 701 Digital Drive Suite J, Linthicum, MD 21090). Another instrument that was used was a Varian CP-3800 GC with a CTC CombiPAL autosampler and a pulsed FPD detector. Other instruments may give equivalent results. General parameters for the Agilent instrument are given in Table 3, and parameters for the Varian instrument are given in Table 4. Parameters can be adjusted for different types of measurements or different analytes, as long as the runs using the parameters are demonstrated to give acceptable results using QC samples with authentic standards.

11.11. Confirmation: Tentative identification of an analyte occurs when a peak from a sample extract falls within the retention time window. Confirmation is necessary when the sample composition is not well characterized. Confirmatory techniques such as gas chromatography with a dissimilar column or a mass spectrometer in Scan mode should be used. When results are confirmed using a second GC column of dissimilar stationary phase, the analyst should check the agreement between the quantitative results on both columns once the identification has been confirmed. When the dual-column approach is employed, the target compounds are identified and confirmed when they meet the identification criteria on both columns. When an MS detector is used, the processing with a retention time and an extracted ion chromatogram is usually sufficient for confident identification of the analytes, unless the chromatogram is excessively complex with overlapping peaks.

Table 3: General instrument parameters for Headspace GC/MS analysis using Gerstel MPS2 autosampler for analysis of CW agent GD used for the method development and validation.

Instrument parameter	**Value**
Headspace vial volume	10 ml or 20 ml
Syringe volume	1.0-2.5 ml
Injection volume	0.25-1.0 ml (depending on sensitivity)
Syringe temperature	75°C
Agitator temperature	40°C
Incubation time	0.5-5 min.
Number of syringe pumps	1-3
Syringe delay (pullup, preinjection, postinjection)	10 sec.
Injection port temperature ramp	40°C (1.5 min.) to 250°C (6.5 min.) at 5°C/sec
Injection/Fill speed	50 µl/sec
Injector penetration of needle	30 mm
Vial penetration of needle	15 mm
GC column	Agilent DB-5MS, 30 m × 0.25 mm × 1 µm film, Cat. No. 122-5533, or similar
GC ramp	35°C (1.5 min) to 250°C (1 min) at 15°C/min.
Carrier gas flow	1.0-1.5 ml/min. He
Solvent delay	5 min.
MS acquisition mode	Scan or SIM (depending on sensitivity)
MS scan rate	2.78 scans/sec
MS ionization mode	EI
Mass range for Scan	40-300 D
Tuning	Standard autotune
MS Source Temp.	230°C
MS Quadrupole Temp.	150°C
Aux-2 Temp.	280°C
Retention Time of GD	10.83 and 10.88 min. (for two diastereomer peaks), or obtain using standards
Quantitation ion(s) for GD	99 and 126 D

Table 4: General instrument parameters for Headspace GC analysis using CTC CombiPAL autosampler and Varian CP-3800 GC for analysis of CW agent GD used for the method development and validation.

Instrument parameter	**Value**
Headspace vial volume	10 ml or 20ml
Syringe volume	1.0 ml
Injection volume	0.1-0.5 ml (depending on sensitivity)
Syringe temperature	50°C
Agitator temperature	40°C
Incubation time	0.5-5 min.
Number of syringe pumps	3
Syringe delay (pullup, preinjection, postinjection)	2 sec.
Split ratio	1:5 to 1:100, depending on sensitivity (A split ratio of at least 1:5 is needed to vent the high volume injection.)
Injection port type	1079 (Variable temperature)
Injection port temperature ramp	60°C (1 min.) to 200°C (3 min.) at 200°C/min (A constant temp. of 200°C can be used to reduce sensitivity by using a split injection to vent some of the analyte to the split flow.)
Injection/Fill speed	100 µl/sec
GC column	Agilent DB-5MS, 30 m × 0.25 mm × 1 µm film, Cat. No. 122-5533, or similar
GC ramp	50°C (3 min) to 200°C (2 min) at 15°C/min.
Column flow	1 ml/min He
Pressure pulse	25 psi for 1 min. (optional)
FPD gases	Fuel=H_2, oxidizer=air
Air1 flow	17 ml/min
Air2 flow	8 ml/min
Hydrogen flow	13 ml/min
Threshold	200 mV
Gate range	6-20 msec for S, 4-10 msec for P
Optical Filter	P or S filter for GD, S filter for HD
PMT gain	510 V
Square root mode	off

12.0 DATA ANALYSIS AND CALCULATIONS

12.1. Sample calculations are given for the spike amounts for film permeation measurements. Calculations for other measurements are similar.

12.1.1. Film area: The inner vial can be used to test a fabric area of 6 mm in diameter. The circle of test fabric can be larger than this size, but it will not be exposed from the agent

vapor in the small vial since it is sealed against the vial glass. The actual exposure area is limited by the I.D. of the glass vial as the seal around the glass lip of the vial prevents contact between the vapor and the fabric. For a 6 mm diameter sample (radius 3 mm), the fabric area is

$$\text{Area} = \pi r^2 = 28.3 \text{ mm}^2 = 0.283 \text{ cm}^2.$$

12.1.2. Agent spike amount: For an agent exposure dose of 1 mg/cm^2, the agent amount for exposure of the fabric would be

$$\text{amount} = \text{dose} \times \text{area} = 1 \times 0.283 = 0.283 \text{ mg}$$

For neat GD, the density is 1.0 g/ml, so 0.283 mg is 0.283 µl of neat liquid agent. It can be difficult to accurately transfer that amount of liquid without specialized equipment.

12.1.3. Agent spike amount from dilute solution: The same dose can be spiked using a dilute solution of GD. If the GD solution is 1000 µg/mL (ppm) = 1 mg/ml, then the amount of solution used in the spike is

$$\text{Spike solution} = 0.283 \text{ mg}/1 \text{ mg-ml}^{-1} = 0.283 \text{ ml} = 283 \text{ µl}$$

As noted previously, this amount of agent would be a large amount for the detector, and the detector could saturate, unless the film that is being tested is quite impermeable so that little agent passes through the film into the large vial.

12.2. Calculations for analysis results

12.2.1. Qualitative determinations or relative comparisons of reactive and unreactive samples: For this determination, a ratio of the signal of the reactive and unreactive samples is calculated, if the detector has a linear response.

$$\text{Relative comparison} = \text{signal(reactive)}/\text{signal(unreactive)}$$

If the detector isn't linear, the calibration curve is used to convert signal to amount, and the ratio is

$$\text{Relative comparison} = \text{amount(reactive)}/\text{amount(unreactive)}$$

Results are reported as the relative comparison, for the spike amount (weight/area or weight/weight) and the reaction time.

12.2.2. Calibration curves: Curves are plotted as signal response for calibration standards vs. amount, at an equilibration temperature. A regression fit is calculated along with a correlation coefficient. The best fit equation is used to calculate amounts from the signal response from sample analyses.

12.2.3. Quantitative determinations: Using the calibration curve and the injection volume, the vapor concentration of the analyte in the sample vial can be determined:

Vapor concentration = amount/volume

The vapor concentration can yield the absolute amount of analyte in the volume of the vial. However, for less volatile agents and high amounts, the amount of agent in the vapor may be saturated. The calculation requires the knowledge of the saturated vapor pressure of the analyte at the equilibration temperature. If the vapor is saturated, then some of the agent is present as liquid, so a calculation of the vapor concentration will not account for all the agent that is present.

12.2.4. Relation to IDLH (Immediately Dangerous to Life and Health vapor concentration): The vapor concentration can be calculated as a multiple of the IDLH (Immediately Dangerous to Life or Health concentration as a 30-min. time-weighted-average exposure). The IDLH for GD = 0.05 mg/m^3.[4] To use more convenient units, 1 $m^3 = 10^6$ cm^3 and 1 mg = 10^6 ng, so the IDLH for GD = 0.05 ng/cm^3 = 50 pg/cm^3. Since a typical sampling volume is 1 ml=1 cm^3 for a headspace GC/MS measurement, it may be difficult to make the sensitivity for the instrumentation described in the method development to be sufficiently sensitive to detect the IDLH concentration. A more appropriate method for sampling to the IDLH is using sorbent tubes which can sample larger volumes of air before being desorbed and injected onto the GC/MS or other suitable type of detector.

However, a variable in this comparison is the fabric area to vial volume that is used. For a fabric measurement, the area of the fabric is known, but the container volume is arbitrary, relative to the IDLH concentration that is relevant to exposure of a final user of the fabric. For these measurements, the fabric is typically in a 10- or 20-ml volume sample vial. Increasing the amount of fabric in the vial, for example to 1 m^2 if possible, would increase the vapor concentration per fabric area by a corresponding amount, for a fixed dose/area amount, so that it would be easier to detect. A vapor concentration that is less than the IDLH target concentration of 50 pg/cm^3 for a small area of fabric is higher for a larger area of fabric in the vial. However, this applies only to reactivity studies, not to permeation studies that are limited by the size of the inner vial rather than the outer vial.

In general, results must be reported in units commensurate with their intended use and all dilutions must be taken into account when computing final results.

13.0 METHOD PERFORMANCE

The performance data in this section are examples of what might be achieved and the data are not intended to be used as acceptance criteria. Method detection limit results are presented as examples only, and each type of material should independently characterized. Sensitivity data is given only as a generic description of anticipated method sensitivity for the example matrix. The information is provided as guidance only, and such limits are highly-matrix dependent and not always achievable. Separate reports are used for actual data on specific matrices.

13.1 Kinetic data for reactivity of solid samples with CW agents: An example is given of the relative reactivity of a treated composite fabric vs. a untreated fabric. The treated composite fabric is PVAM Dark/Cleanshell Tough, received from Natick on 23 Nov. 2010. The untreated fabric is untreated NyCo fabric.

Figure 7 shows the total ion chromatogram of the headspace GC/MS analysis of fabric spiked with GD. The GD has a characteristic doublet due to the two diastereomers. Figure 8 shows the mass spectrum.

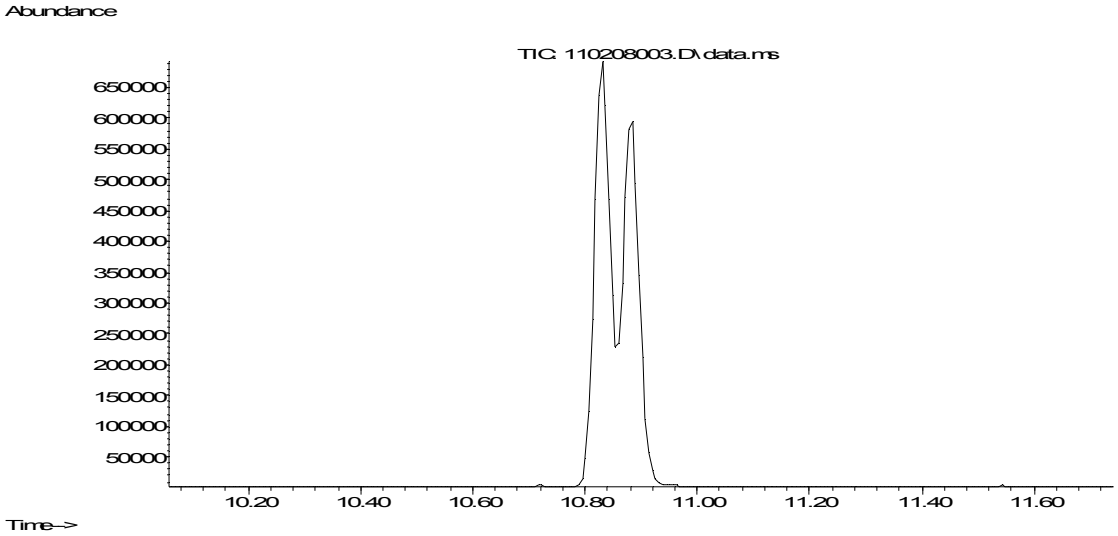

Figure 7: Total Ion Chromatogram from blank fabric sample NB214P88G spiked with GD, after sitting at room temperature for 24 hrs.

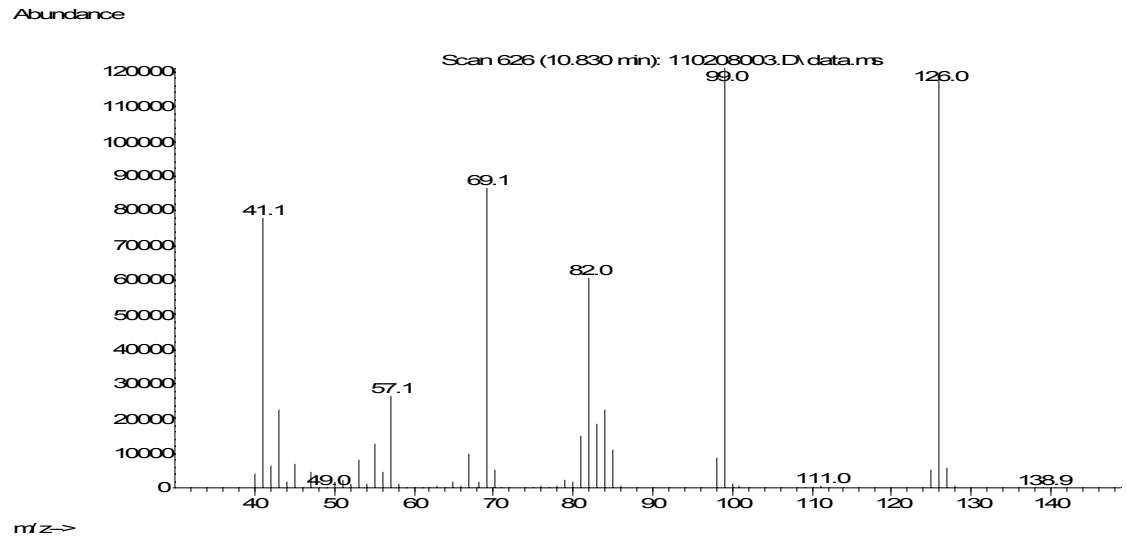

Figure 8: Mass Spectrum from the peak of the chromatogram of NB214P88G shown in Figure 7.

The samples were run using full scan mass spectra, since optimal sensitivity was not a goal, but the sensitivity could be improved by using Selected Ion Monitoring on the 126 and 99 D ions. For integrating the peaks, the scan spectrum is can be processed to extract a particular ion signal. Figure 9 shows the extracted ion signal for the 126 D ions to compare the relative signals for an untreated fabric and the treated composite fabric.

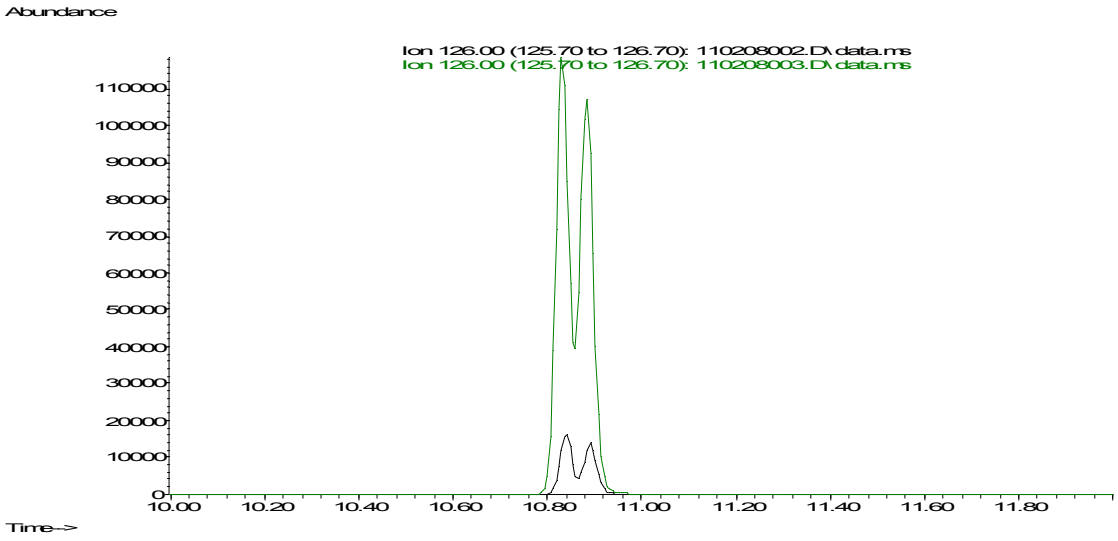

Figure 9: Overlaid extracted ion chromatograms for 126 D ion, for samples P88A (Treated fabric, lower signal trace) and P88G (untreated fabric, larger signal trace). The integrated area of the treated composite fabric is 12.6% of the area of the untreated fabric.

Table 5 shows the comparison of three pairs of treated and untreated fabrics that were spiked at the same time and analyzed at different times. Figure 10 shows the data points, normalized to 1.0 for the point at 24 hrs, and compared to NMR kinetic data for the same composite fabric. The agreement is good between the two methods for the slope of the plot. However, the Headspace GC/MS method indicates that the GD is almost undetectable in 168 hrs, while the NMR method indicates that GD is still present. The amount of GD that was spiked on the fabric is much less for the Headspace method than it was for the NMR method: the amount of GD was 50 µg (in dilute solution) for the Headspace method, and 1 mg (neat) for the NMR method.

Table 5: Reactivity of GD on PVAM Dark/Cleanshell Tough, received from Natick on 23 Nov. 2010. Samples P88A, P88B, and P88C are replicate identical samples that were analyzed at the time shown after spiking. The untreated NyCo fabric are samples P88G, P88H, and P88I.

Decrease of signal on fabric rel. to blank fabric vs. time , humidified at 80%RH

file	sample	time (hr)	no. inj.	126 signal (K)	Rel.
110208003	NB214P88G	24	1	4567	
110208002	NB214P88A	24	1	578	0.12656
110210005	NB214P88H	72	1	3549	
110210004	NB214P88B	72	1	207	0.058326
110214005	NB214P88I	168	1	1605	
110214008	NB214P88C	168	1	2	0.001246

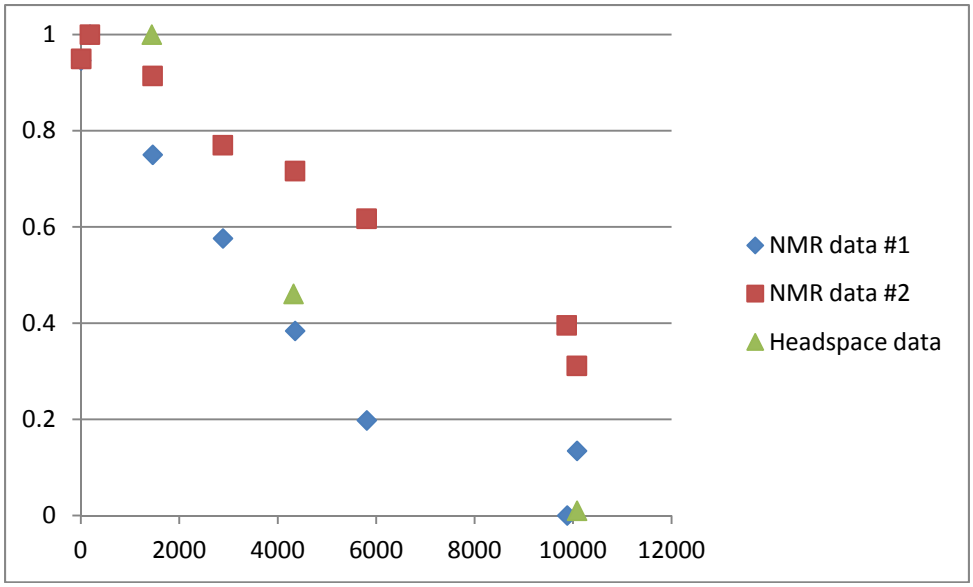

Figure 10: Comparison of relative kinetic data for Headspace GC/MS and two NMR runs for the same composite fabric. The y-axis is linear and plotted so the highest point is scaled to 1.0.

13.1.1. Repeated sampling of the same sample vial: Efforts were made to measure kinetics by repeated sampling of the same vial. In general, this approach may provide acceptable results for short studies (<24 hrs).

13.1.2. Low volatility CW agents: CW agents with low volatility can be detected by this method by using elevated agitator and syringe temperature. For example, VX was detected. Kinetics data was not measured for reaction of low volatility agent with a solid sample because a suitable reactive solid sample was not found. It is not known whether accurate kinetics can be obtained for a low volatility agent by using an elevated agitator temperature, since the solid sample will be heated and the heating may alter the reactivity. For the vapor permeation studies, the exposure of the fabric to agent vapor will be limited by the low volatility of the agent.

13.2. Qualitative determinations or relative comparisons of reactive and unreactive samples: Analysis of chloramide fabrics by Headspace GC/MS method.

The following fabrics were tested in parallel:
1. April 2010 Cleanshell-light fabric treated with BA-1 chloramide and Quat. (quaternary amine biocide)
2. April 2010 Cleanshell-light that was treated with BA-1 chloramide, Quat., and a fluorosilane repellent coating
3. As a control, an untreated NyCo fabric was tested.
4. 2009 sample from Tyndall AFB labeled RAS090105B treated with BA-1 chloramide
5. 2009 fabric sample labeled Tyndall+UC State treated with BA-1 chloramide

All samples except #3 received nominally identical treatments that should react oxidatively toward HD.

Samples 1-4 were rechlorinated to reactivate all chloramide groups, including the untreated NyCo fabric. Fabric samples were soaked in 5% sodium hypochlorite solution (commercial bleach) for 30 min. then rinsed 30 sec. under running DI water and air dried. Samples 1-3 were rechlorinated on 8/26/10 and reacted a month later. Sample 4 was rechlorinated the day before reaction. Sample 5 was not rechlorinated and it was used as-is from the storage bag.

The five fabric samples were run using the Headspace GC/MS method. A fabric square of 1 cm^2 was placed in a 10 mL headspace vial. The fabric was spiked with 50 µL of 1 mg/mL solution of HD in chloroform-d, for a spike amount of 50 µg. The vials were uncapped in a hood for 20 min. to allow the solvent to evaporate. The vials were capped and allowed to react at room temperature for 24 hrs. They were run by headspace GC/MS using an automated sampling syringe. They were incubated at 40°C during sampling, and the syringe was heated to 75°C. Two replicate injections of the same set of vials were run.

Results show that the amount of HD that is detected for the two 2009 fabrics is 100 times less than the April 2010 fabrics. The amount of HD for this measurement for the April 2010 fabrics was 23-34% of the amount for untreated fabric.

Table 6: Reactivity of HD + chloramide fabrics by Headspace GC/MS. Integrated areas for GC/MS ion signals (m/z 109) for HD are given.

		Extracted ion signals for HD	Relative Recoveries	
file	sample no.	m/z 109 area (in Kcounts)	m/z 109	Notes on sample type
100930001	50 ug std.	11673	218.5%	50 ug in an empty vial
100930002	NB214P69B	5343	100.0%	50 ug on untreated fabric
100930003	NB214P69C	1539	28.8%	50 ug on 4/7/10 chloramide
100930004	NB214P69D	1818	34.0%	50 ug on 4/7/10 chlor/repell
100930005	NB214P69E	22	0.4%	50 ug on 2009 chloramide
100930006	NB214P69F	6	0.1%	50 ug on 2009 UNC/Tyndall
100930007	50 ug std.	10540	197.3%	50 ug in an empty vial
100930008	NB214P69B	3099	58.0%	50 ug on untreated fabric
100930009	NB214P69C	1213	22.7%	50 ug on 4/7/10 chloramide
100930010	NB214P69D	1602	30.0%	50 ug on 4/7/10 chlor/repell
100930011	NB214P69E	20	0.4%	50 ug on 2009 chloramide
100930012	NB214P69F	7	0.1%	50 ug on 2009 UNC/Tyndall

Low reactivity of the 2010 fabrics was confirmed by NMR experiments and by a simple qualitative test for oxidation using potassium iodide solution.

13.2.1. Repeated sampling of the same sample vial: Each vial was remeasured in this experiment. Results for the second measurement were similar to the first measurement, and the results give an indication of the uncertainty in the measurement.

13.2.2. Qualitative vs. Quantitative interpretation: These results give qualitative information, showing the relative reactivity of the fabrics from 2009 is greater than the fabrics from 2010. Extraction of quantitative information is more difficult, since it depends on several additional factors. It is interesting that the signal above the spiked unreactive fabric is only 50% compared to the same amount of agent in the empty vial. This result could indicate that the agent is bound to the unreactive fabric. In an independent study, it was found that the vapor above a CARC paint sample was only about 1% of the expected amount from the spike amount.

This effect indicates that even untreated fabrics absorb a significant amount of agent, dependent on the agent and the solid material. This issue was not studied as part of this method development.

13.3. Calibration curve: A calibration curve was measured for agent in empty vials, shown in Table 7. Note that the data in Table 7 is not directly comparable to Table 6, since Table 7 data was integrated for the Total Ion Chromatogram, and for Table 6 the integral was for the Extracted Ion Chromatogram. Also, the method was altered to be more sensitive. The calibration curve for the data is shown in Figure 11: Calibration curve and best fit polynomial for headspace data in Table 7.. The curve is fit best to a quadratic polynomial, and the curve gives a correlation coefficient of 0.995.

Since the curve doesn't saturate, it is likely that the HD vapor in the vial is not saturated, and all the HD is vaporized, for these spike amounts.

Table 7: Calibration data for HD in empty headspace vials.

File ID	Volume (uL)	% HD (v/v)	HD (ug)	RT (min)	TIC Area
09082416.d	1	0.01	0.127	12.861	2,156,688
09082417.d	2	0.01	0.254	12.861	6,165,519
09082418.d	4	0.01	0.508	12.860	12,197,645
09082419.d	6	0.01	0.762	12.861	22,193,337
09082420.d	8	0.01	1.016	12.861	34,431,387
09082421.d	1	0.10	1.27	12.860	37,931,584
09082422.d	3	0.10	3.81	12.865	116,089,721
09082423.d	5	0.10	6.35	12.871	280,357,649
09082424.d	7	0.10	8.89	12.875	408,431,180

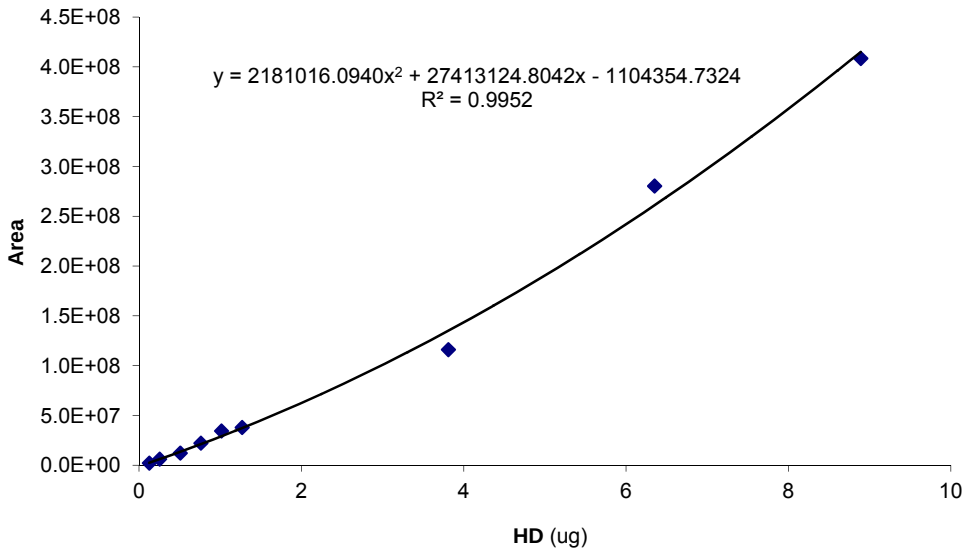

Figure 11: Calibration curve and best fit polynomial for headspace data in Table 7.

13.4. Polymer or other solid material reaction: Relative kinetic data can be obtained by spiking CW agent or simulant on a solid reactive polymer. The data in Figure 12 was taken by multiple sampling of two vials over 16 hrs. The data is normalized to the maximum point for each data set. NMR kinetic data is included for comparison.

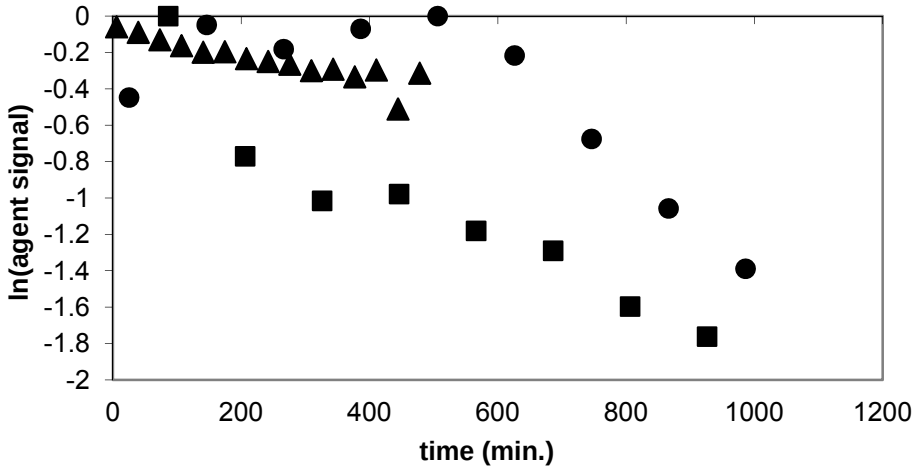

Figure 12: Comparison of kinetic data on solid PANOx polymer, log of signal vs. time. ●: DFP on PANOx using Headspace GC/MS, ▲ NMR data for GD on PANOx, ■ GD on PANOx using Headspace GC/MS. Data is normalized to the maximum point.

13.5. Film permeation: Following the vial-in-vial procedure in Section 11.2.3 and the diagram in Figure 5, the permeation through a film was measured. Example data is shown in Figure 13. Static Permeation of HD is shown through films from a nitrile glove, chloramide fabric, and CARC paint. The vial cap is used as a negative control. The permeation through this sample of the chloramide fabric may be due to the loose weave of the fabric and wettability, so that the agent is easily transferred through the fabric to the outside vial. Nitrile gloves are well known to be fairly permeable to agent once they are exposed. Relative kinetic data was obtained by multiple resampling of the same vials.

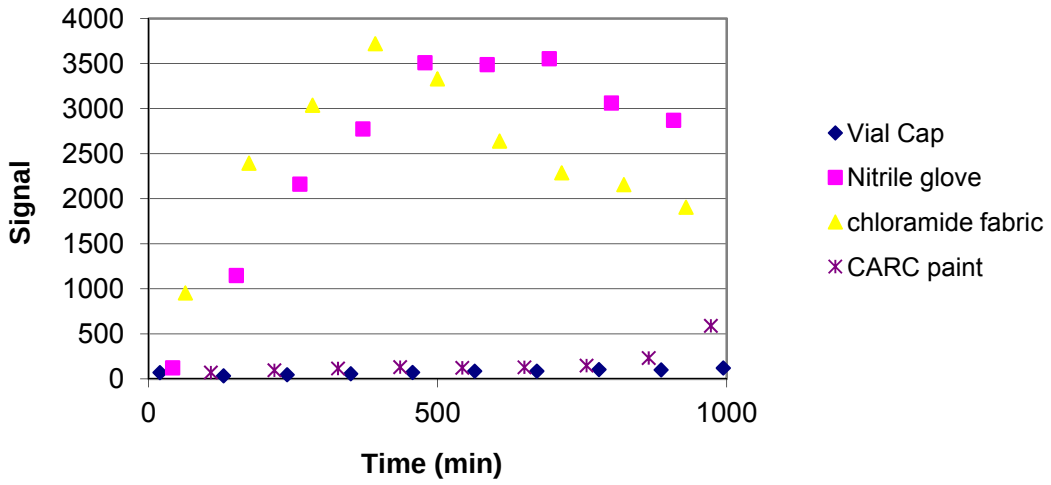

Figure 13: Static Permeation of HD through films from a nitrile glove, chloramide fabric, and CARC paint. The vial cap is used as a negative control.

13.6 Performance data and related information are provided in methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

14.0 REFERENCES

[1] U.S. EPA METHOD 5021A, "VOLATILE ORGANIC COMPOUNDS IN VARIOUS SAMPLE MATRICES USING EQUILIBRIUM HEADSPACE ANALYSIS,"
http://www.epa.gov/osw/hazard/testmethods/pdfs/5021a_r1.pdf

[2] Vapor pressure of HD was obtained from E. C. Penski, "Properties of Di-(2-Chloroethyl) Sulfide 1. Vapor Pressure Data Review and Analysis," Edgewood Research, Development, and Engineering Center Technical Report ERDEC-TR-043, Aberdeen Proving Ground, MD, April 1993. Partial pressure was calculated from the mole ratio.

[3] R. J. O'Connor, M. D. Brickhouse, D. McGarvey, H. D. Durst, W. R. Creasy, and J. L. Ruth, *NMR Method for the Quantitative Purity Analysis of Neat Feed Stock Samples,* ECBC Technical Report ECBC-TR-253, Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, Aug. 2002.

[4] R. J. Mioduszewski, S. A. Reutter, L. L. Miller, E. J. Olajos, and S. A. Thomson, Evaluation of Airborne Exposure Limits for G-Agents: Occupational and General Population Exposure Criteria, Edgewood Research, Development, and Engineering Center Technical Report ERDEC-TR-489, Aberdeen Proving Ground, MD, April 1998.

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